2023-2024 CALIFORNIA LEAFY GREENS RESEARCH PROGRAM
ANNUAL REPORT

Project Title: Characterizing the accumulation of impatiens necrotic spot virus (INSV) in lettuce tissues and incidence in thrips vectors.

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Abstract

Experiments are examining how impatiens necrotic spot virus (INSV) accumulates in different tissues of lettuce plants, how this varies among susceptible and tolerant or resistant varieties, and how this may influence the development of thrips populations that can carry the virus to new plants. Studies are comparing accumulation of INSV in field-grown lettuce with how the virus accumulates in lettuce plants inoculated under controlled conditions with standardized methods in order to standardize comparison among romaine varieties that differ for perceived resistance or tolerance to INSV or that have been shown in preliminary studies to exhibit variable tissue-specific virus accumulation. INSV was inoculated to sets of lettuce seedlings of each of several lines using viruliferous thrips. Infections were allowed to develop in growth chambers. INSV accumulation was measured in leaves, crown tissue and roots of plants using serological testing (ELISA) with antiserum against INSV. Much of this first year has involved optimization of methods as infections have not been consistent until recently. Once fully optimized, phase 2 will involve collection of individual adult thrips from each plant and evaluation of thrips for the presence of INSV. This will provide information on how plants exhibiting known differences in INSV distribution may influence virus spread in a field by determining virus incidence in thrips populations derived from lettuce differing in tissue-specific virus accumulation.

Summary of previous accomplishments from this project relevant to this year's research:

- Demonstrated that INSV infects root tissues in addition to leaf tissues in susceptible lettuce varieties (SB170 funding, ongoing).
- Observed differential accumulation of INSV in root, crown, and leaf tissues in several lettuce varieties (SB170 funding, ongoing).
- Determined need for inoculation using larger thrips numbers and additional control inoculations due to infection rate issues. Adjustments were made to experimental design and new experiments are in progress.

Objectives

1. *Determine the distribution and accumulation of INSV in lettuce varieties that are considered resistant or tolerant and assess their impact on plant quality.* The work will provide information on where INSV is accumulating in lettuce plants, which will influence the perception of INSV-resistant breeding lines and inform future breeding efforts.

2. *Assess the potential for INSV to be acquired by thrips from lettuce cultivars exhibiting altered virus distribution patterns.* This will provide knowledge as to how the distribution and accumulation of INSV in lettuce plants affects thrips acquisition of the virus from the host plant.

Procedures

Objective 1. *Determine the distribution and accumulation of INSV in lettuce varieties that are considered resistant or tolerant and assess their impact on plant quality.*

Studies are specifically comparing INSV distribution and accumulation in different portions of lettuce plants (leaves, crown, roots) of a known susceptible variety (Parris Island) with known tolerant or resistant romaine lettuce varieties. (Smith et al., 2022; Hasegawa unpublished). The studies by Smith et al. (2022) demonstrated that crisphead lettuces in general had lower INSV severity overall than romaine lettuces. Therefore, our initial studies are focused on romaine varieties.

Using established protocols by the Hasegawa lab, western flower thrips at the first instar larval stage from reared colonies at USDA-ARS in Salinas, CA are transferred to detached INSV-infected leaves to acquire the virus for 24 hr. Larvae are then maintained to allow completion of their life cycle to the adult stage. Within 48 hrs of adult emergence, viruliferous adult thrips are collected (numbers have ranged from two to fifteen in efforts to optimize infection), thrips are placed into a clip-on cage, and transferred to leaves of young lettuce plants at the first to second true leaf stage to allow for virus transmission. Plants are maintained in thrips-proof cages in a growth chamber for six weeks.

At two, four, and six weeks post initial exposure to viruliferous thrips (thrips carrying INSV), individual lettuce plants of each set of plants are scored for symptoms using a visual severity scale of 0-5 (Hasegawa and Del-Pozo, 2022). Initial plans intended that three plants per line will be sacrificed and roots washed free of soil at each time point; however, infection numbers have been low until recently. Therefore, plants were not sacrificed at 2 and 4 weeks, rather only at six weeks as we optimize methods. The earlier time points will be included again moving forward now that infection is becoming more consistent with improved methodology. Samples are collected separately from leaf, crown, and root of each plant and subjected to TAS-ELISA (serological virus detection method) using INSV-specific antiserum (Agdia, Elkhart, IN) to determine incidence and titer of virus in each tissue section. Results will be analyzed using linear regression with post hoc tests for differences in virus accumulation and distribution in plant tissue, but to date we have not had sufficient consistency among experiments for such an analysis.

Objective 2. *Assess the potential for INSV to be acquired by thrips from lettuce cultivars exhibiting altered virus distribution patterns.*

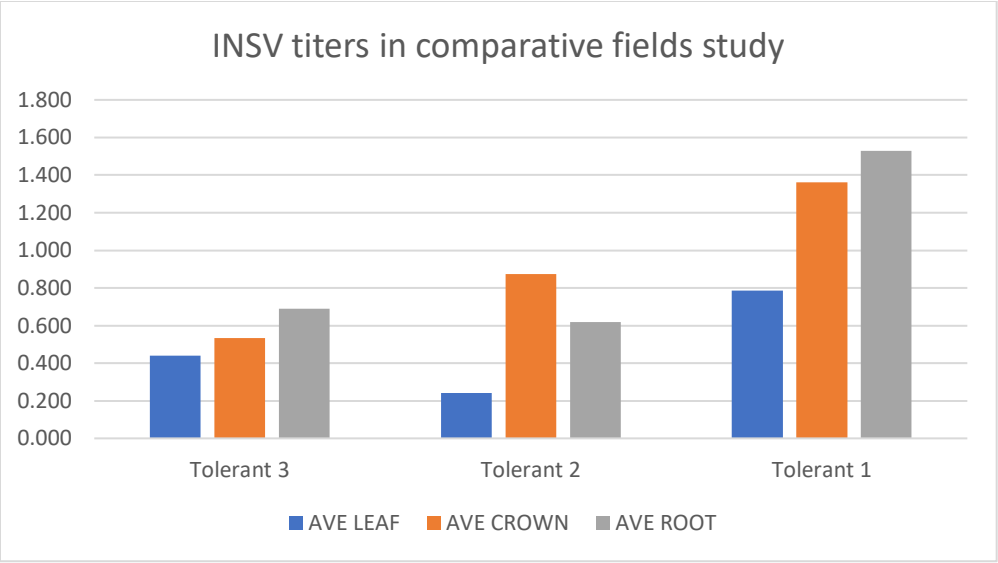
Based on the outcomes of Objective 1, two varieties that show the greatest differences in virus distribution and accumulation patterns will be used to evaluate their potential as hosts from which thrips can acquire the virus. Following full development of INSV symptoms in the susceptible line, 20 adult thrips will be added to each plant of each variety (3 plants per variety) to allow egg laying for 1 week. At that point, adults will be manually removed from the plants and eggs that were laid in the plants will be given 2 weeks to develop to the adult stage. Five adult thrips will be collected from each plant and tested for the frequency at which they are infected with INSV. Total RNA will be extracted from individual thrips using methods established by Hasegawa and will be tested for INSV using reverse-transcription quantitative PCR (RT-qPCR). The frequency of INSV infection will be compared between the susceptible and tolerant varieties to assess their risk as hosts from which thrips can acquire INSV and differences determined by a one-way analysis of variance.

Results

Field studies were conducted during fall 2023, and INSV accumulation among leaf, root, and crown tissues was compared for some of the same varieties as in controlled growth chamber experiments. Symptoms in these experiments were very mild, overall, with only a few plants showing severe symptoms. This was believed to be the result of milder than usual INSV incidence during fall 2023 rather than a full reflection on performance of the tolerant varieties, based on observations. Field results supported earlier preliminary studies indicating that when plants became infected, virus accumulated to higher titers (amount of virus per amount of tissue sample) in root and

crown tissues of tolerant varieties than in leaves (Fig. 1), although differences were not as pronounced as in the preliminary studies.

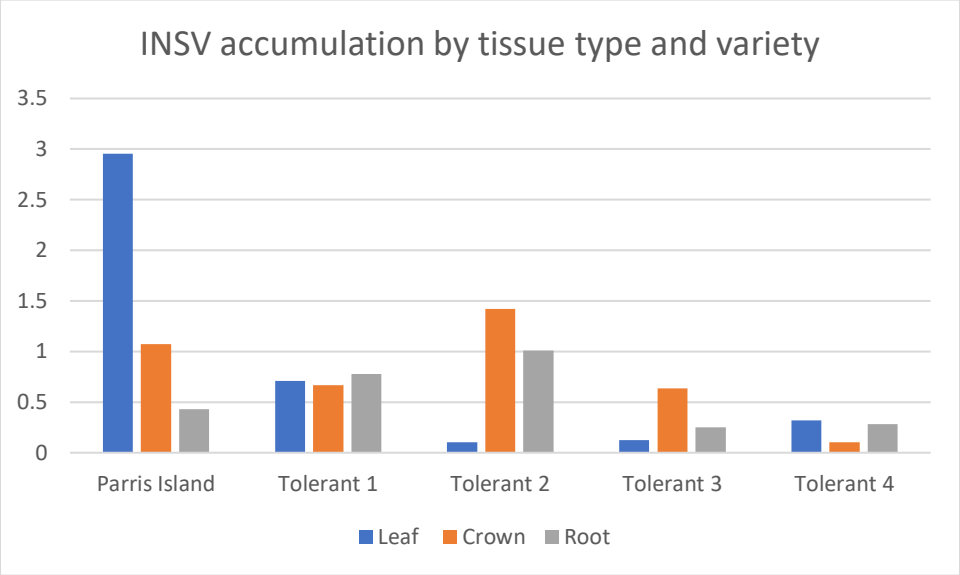
Figure 1. INSV titer in field grown lettuce (October 2023)



Results of growth chamber studies were much more variable. In most cases leaf tissue did show lower virus titers than root and crown, but this was not observed in all cases, and two varieties showed highest accumulation in the crown. Importantly, INSV titer in leaf tissue was generally lowest of the three tissue types regardless of variety (Fig. 2), supporting the expectation that these varieties show reduced virus accumulation in leaves, and also supporting our hypothesis that the thrips should have reduced access to virus in leaf tissues of tolerant varieties.

Research on thrips acquisition and transmission from infected lettuce has been delayed pending optimization of transmissions, as we have had low numbers of positive plants in most experiments until recently, and most plants have been sacrificed in experimentation to evaluate virus titer among tissues. Since the last two experiments have had better infection rates, we plan to proceed with Objective 2 during late summer and fall 2024.

Figure 2. INSV titer as measured by absorbance in ELISA tests by coded variety



Discussion

This project is providing a standardized evaluation of the performance of INSV-tolerant romaine lettuce varieties for accumulation of INSV in different plant tissues (leaf, crown, and root), and an associated evaluation of how virus titer in leaf tissue may influence thrips acquisition of INSV and subsequent transmission to new lettuce plants. This information will be of value to the lettuce industry by demonstrating how suppression of virus titer in plants may influence epidemiology and spread within and among lettuce fields.

The project has had a number of challenges in its early stages, with the most impactful being low rates of infection under controlled conditions. This has slowed progress as modifications to methods were necessary to improve transmission rates under controlled conditions. In particular, the original plan of using two adult thrips per plant for inoculations was not reliable as initial experiments had few infected plants. We later increased numbers to 10 per plant, but that also remained inconsistent and suggested that acquisition of INSV by larval thrips was not consistent or reliable. Otherwise, the increased numbers should have led to much higher infection rates of test plants. Virologists use a term, “acquisition access period” to indicate that an insect vector is given a certain amount of time on which it has access to feed on an infected plant; however, simply having access to the food source does not mean the insect feeds steadily or efficiently. It is suspected that although the thrips are given access to virus-infected leaf tissue for 24 hours, they may feed enough to stay alive, but may not be feeding efficiently enough to effectively acquire enough virus to allow the virus to build up in the insect as it matures. INSV is acquired by first instar (youngest) thrips larvae, then accumulates and replicates in the insect. INSV can only be transmitted to plants by

an adult thrips. To adjust our acquisition efficiency, we switched virus acquisition and inoculation to greenhouse conditions, then moved inoculated plants to the growth chamber for standardization of test conditions. This has been far more effective, although it does not fully standardize the methods as we had originally intended. That being said, by keeping all plants under the same conditions, we still have standardization.

Now that we are starting to see sufficient numbers of infected plants, we will begin more in-depth analyses, which had been delayed due to low numbers of infected plants. This will allow evaluation of tissue-specific virus accumulation as well as subsequent studies under objective 2 to allow us to determine how effectively thrips acquire and transmit INSV from tolerant lettuce. As noted above, generally tolerant lettuce varieties show lower accumulation in leaf tissue than is found in leaf tissue from susceptible lettuce.

One additional modification that may be necessary is that we will sample leaf tissue prior to thrips acquisition testing, as there is clearly some variability even among tolerant varieties for accumulation in leaf tissue. One leaf will be sampled for ELISA testing to determine virus titer, whereas a comparable leaf on the same plant will be used for thrips acquisition.

Although progress has been delayed, optimization efforts over the first year have led to improvement in approaches, that should lead to completion by spring of 2025.

References

1. Koike ST, Kuo YW, Rojas MR, and Gilbertson RL. 2008. First Report of *Impatiens necrotic spot virus* Infecting Lettuce in California. *Disease Notes.* https://doi.org/10.1094/PDIS-92-8-1248A
2. Hasegawa DK and Del-Pozo A. 2022. Epidemiology and Economic Impact of Impatiens Necrotic Spot Virus: A Resurging Pathogen Affecting Lettuce in the Salinas Valley of California. *Plant Disease.* https://doi.org: 10.1094/PDIS-05-22-1248-RE
3. Hasegawa DK, Hladky LJ, Wintermantel WM, Putman AI, Barman A, Slinski S, Palumbo J, and Poudel-Ward B. 2022. First Report of Impatiens Necrotic Spot Virus Infecting Lettuce in Arizona and Southern Desert Regions of California. https://doi.org/10.1094/PDIS-09-21-2118-PDN
4. Simko I, Richardson CE, Wintermantel WM. 2018. Variation within *Lactuca* spp. for resistance to *Impatiens necrotic spot virus. Plant Disease.* 102: 341-348; doi:10.1094/PDIS-06-17-0790-RE
5. Smith R, Dundore Arias JP, Richardson K, Wang Y-C, and Love T. 2022. 2022 Lettuce variety tolerance to pythium wilt. Salinas Valley Agriculture, Dec. 2, 2022. UCANR. https://ucanr.edu/blogs/blogcore/postdetail.cfm?postnum=55733